

Technologies, Inc.) in Opti-MEM, serially diluted to the desired concentrations, and applied to washed cells. Basal and untreated (no oligonucleotide) control cells were also treated with Lipofectin. Cells were incubated for 4 h at 37 °C, at which time the medium was removed and replaced with standard growth medium with or without 5 mg/mL TNF- $\alpha$  7 & D Systems). Incubation at 37 °C was continued until the indicated times.

***Quantitation of ICAM-1 Protein Expression by Fluorescence-activated Cell Sorter***

[0273] Cells were removed from plate surfaces by brief trypsinization with 0.25% trypsin in PBS. Trypsin activity was quenched with a solution of 2% bovine serum albumin and 0.2% sodium azide in PBS (+Mg/Ca). Cells were pelleted by centrifugation (1000 rpm, Beckman GPR centrifuge), resuspended in PBS, and stained with 3 l/10<sup>5</sup> cells of the ICAM-1 specific antibody, CD54-PE (Pharmingen). Antibodies were incubated with the cells for 30 min at 4C in the dark, under gently agitation. Cells were washed by centrifugation procedures and then resuspended in 0.3 mL of FacsFlow buffer (Becton Dickinson) with 0.5% formaldehyde (Polysciences). Expression of cell surface ICAM-1 was then determined by flow cytometry using a Becton Dickinson FACScan. Percentage of the control ICAM-1 expression was calculated as follows: [(oligonucleotide-treated ICAM-1 value) - (basal ICAM-1 value)/(non-treated ICAM-1 value) - (basal ICAM-1 value)]. (Baker, Brenda, *et. al.* 2'-O-(2-Methoxy)ethyl-modified Anti-intercellular Adhesion Molecule 1 (ICAM-1) Oligonucleotides Selectively Increase the ICAM-1 mRNA Level and Inhibit Formation of the ICAM-1 Translation Initiation Complex in Human Umbilical Vein Endothelial Cells, *The Journal of Biological Chemistry*, 272, 11994-12000, 1997.)

[0274] ICAM-1 expression of chimeric C3'-endo and C2'-endo modified oligonucleotides of the invention is measured by the reduction of ICAM-1 levels in treated HUVEC cells. The oligonucleotides are believed to work by RNase H cleavage mechanism. Appropriate scrambled control oligonucleotides are used as controls. They have the same base composition as the test sequence.

[0275] Sequences that contain the chimeric C3'-endo (2'-MOE)and.C2'-endo (one of the following modifications: 2'-S-Me, 2'-Me, 2'-ara-F,2'-ara-OH, 2'-ara-O-Me) as listed in Table X below are prepared and tested in the above assay. SEQ ID NO: 24, a C-raf targeted oligonucleotide, is used as a control.

**Table X**  
**Oligonucleotides Containing chimeric**  
**2'-O-(2-methoxyethyl) and 2'-S-(methyl) modifications.**

SEQ ID NO:	Sequence (5'-3')	Target
24	<b>AsTsGs C<sup>m</sup>sAsTs TsCs<sup>m</sup>Ts GsCs<sub>m</sub></b> <b>Cs<sup>m</sup> Cs<sup>m</sup>C<sup>m</sup>sC<sup>m</sup>s AsAsGs GsA</b>	mouse C-raf
25	GsC <sup>m</sup> sC <sup>m</sup> s C <sup>m</sup> sAsAs GsC <sup>m</sup> sTs GsGsC <sup>m</sup> s ASTsC <sup>m</sup> S C <sup>m</sup> sGSTs C <sup>m</sup> SA	human ICAM-1

[0276] All nucleosides in bold are 2'-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-S-Me- modification. Superscript m on C (Cm)indicates a 5-methyl-C.

**Table XI**  
**Oligonucleotides Containing chimeric**  
**2'-O-(2-methoxyethyl) and 2'-O-(methyl) modifications**

SEQ ID NO:	Sequence (5'-3')	Target
24	<b>AsTsGs C<sup>m</sup>sAsTs TsCs<sup>m</sup>Ts</b> <b>GsCs<sup>m</sup>Cs<sup>m</sup> Cs<sup>m</sup>C<sup>m</sup>sC<sup>m</sup>s AsAsGs</b> <b>GsA</b>	mouse C-raf

25                    GsC<sup>m</sup>sC<sup>m</sup>s C<sup>m</sup>sAsAs GsC<sup>m</sup>sTs human  
                       GsGsC<sup>m</sup>s ASTsC<sup>m</sup>S C<sup>m</sup>sGSTs ICAM-1  
                       C<sup>m</sup>SA

[0277]            All nucleosides in bold are 2'-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-Methyl modification. Superscript m on C (Cm) indicates a 5-methyl-C.

**Table XII**  
**Oligonucleotides Containing chimeric**  
**2'-O-(2-methoxyethyl) and 2'-ara-(fluoro) modifications**

SEQ ID NO:	Sequence (5'-3')	Target
24	AsTsGs C <sup>m</sup> sAsTs TsCs <sup>m</sup> Ts	mouse
	GsCs <sup>m</sup> Cs <sup>m</sup> Cs <sup>m</sup> C <sup>m</sup> sC <sup>m</sup> s AsAsGs	C-raf
	GsA	
25	GsC <sup>m</sup> sC <sup>m</sup> s C <sup>m</sup> sAsAs GsC <sup>m</sup> sTs	human
	GsGsC <sup>m</sup> s ASTsC <sup>m</sup> S C <sup>m</sup> sGSTs	ICAM-1
	C <sup>m</sup> SA --	

[0278]            All nucleosides in bold are 2'-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-ara-(fluoro) modification. superscript m on C (Cm) indicates a 5-methyl-C.

**Table XIII**  
**Oligonucleotides Containing chimeric**  
**2'-O-(2-methoxyethyl) and 2'-ara-(OH) modifications**

SEQ ID NO:	Sequence (5'-3')	Target
24	AsTsGs C <sup>m</sup> sAsTs TsCs <sup>m</sup> Ts	mouse
	GsCs <sup>m</sup> Cs <sup>m</sup> Cs <sup>m</sup> C <sup>m</sup> sC <sup>m</sup> s AsAsGs	C-raf
	GsA	